Diarylpropionitrile (DPN) Enantiomers: Synthesis and Evaluation of Estrogen Receptor Beta-Selective Ligands

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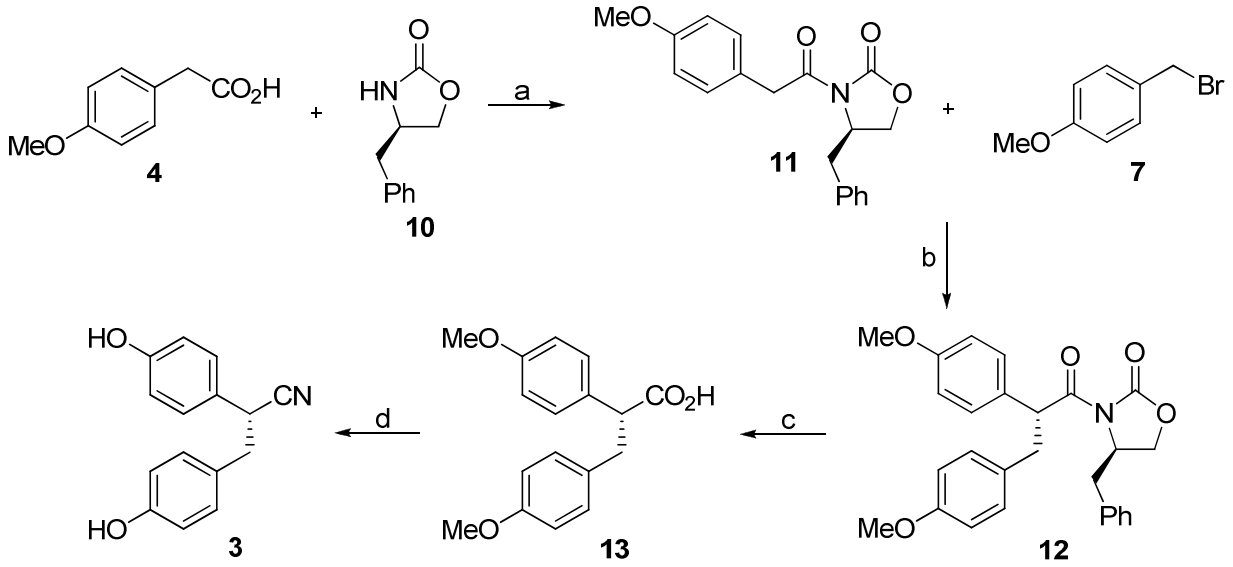
Abbreviations: A, FRET acceptor; CARLA, coactivator recruitment ligand assay; D, FRET donor; DPN, diarylpropionitrile; E_2, estradiol; ER, estrogen receptors; Fl, fluorescein; LBD, ligand binding domain; NRID, nuclear receptor interaction domain; PPT, propyl pyrazole triol; RCA, relative coactivator binding affinity; RLA, relative ligand binding affinity; RCP, relative cellular potencies; RRP, relative recruitment potency; SERMs, selective estrogen receptor modulators; SRC3, steroid receptor coactivator 3; Tb, terbium; tr-FRET, time-resolved resonance energy transfer;

Supporting Information:

Experimental Procedures

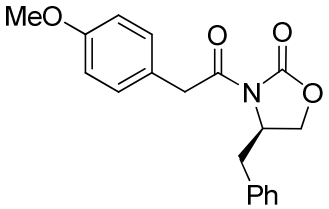
General Procedures: All reactions were carried out under a nitrogen atmosphere with dry solvents using anhydrous conditions unless otherwise stated. THF, DCM and $PhCH_3$ used in the reactions were dried in a solvent delivery system (neutral alumina column). Reagents were purchased from Aldrich and used without further purification, unless otherwise stated. Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials, unless otherwise stated. Reactions were monitored by thin layer chromatography (TLC) carried out on Merck silica gel 60 F254 precoated plates (0.25 mm) using UV light as the visualizing agent and ceric ammonium molybdate and heat as developing agents. Flash column chromatography was performed on Silica P Flash silica gel (40-64 μM, 60 Å) from SiliCycle. ^{1}H NMR spectra were recorded at 23 ^{o}C on a Varian Unity-400, Varian Inova-500 or Varian Unity-500 spectrometers and are reported in ppm using residual protium as the internal standard ($CHCl_3$, δ = 7.26, CD_2HCN, δ = 1.94, center line, acetone-d_6, δ = 2.05, center line). The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, dd = doublet of doublets, t = triplet, q = quartet, m = multiplet and b = broad. Proton-decoupled ^{13}C NMR spectra were recorded on a Varian Unity-500 (126 MHz) spectrometer and are reported in ppm using solvent as an internal standard ($CDCl_3$, δ = 77.16, CD_3CN, δ = 1.30, center line, acetone-d_6, δ = 29.80, center line). High resolution mass spectra were obtained at the University of Illinois Mass Spectrometry Laboratory. The purities of target compounds were ≥95%, measured by HPLC using a Waters 1525 binary HPLC pump equipped with a Waters in-line degasser AF, Waters 2487 Dual λ absorbance detector and a Symmetry C18 5 µm 4.6 X 150 mm column (Part No. WAT045905). Chiral high pressure liquid chromatographic (HPLC) analysis was performed using a Waters 1525 binary HPLC pump equipped with a Waters in-line degasser AF, Waters 2487 Dual λ absorbance detector and a Regis Technologies (R,R)-Whelk-O® 2 column (Particle Size: 10 µm, 100 Å, Column Dimensions: 25 cm X 4.6 mm, Cat. #: 786315). Optical rotations were obtained using a JAS.CO DIP-370 Digital Polarimeter and a 3.5 x 50 mm cell and are reported as follows: concentration (c = g / 100 mL), solvent. Melting points were recorded on a Thomas Hoover Uni-Melt 6427-F10 Capillary Melting-Point Apparatus. [^{3}H]-17β-Estradiol, specific activity 118 Ci/mmol (4366 GBq/mmol) was purchased from Perkin Elmer Life Science (Boston, MA). 17β-Estradiol (17β-E_2) was obtained from Sigma (St. Louis, MO). Purified full-length human ERα and ERβ were purchased from Pan Vera (Madison, WI). The thiol reactive fluorophore, 5-iodoacetamido fluorescein and terbium labeled streptavidin were obtained from Molecular Probes/Invitrogen (Eugene, CA). Thiol reactive biotin derivative (MAL-dPEG4-biotin) was from Quanta BioDesign (Powell, OH).

Scheme 2. Synthesis of *R*-DPN (3)[a]



[a]Reagents and conditions: (a) pivaloyl chloride, TEA, PhMe, reflux; (b) NaHMDS, THF, -78 °C; then **7**, THF, -78 °C→rt; (c) H_2O_2, LiOH, THF:H_2O (5:1), rt; (d) (i) $ClCO_2CH_2CH(CH_3)_2$, TEA, THF, -20 °C; then NH_3 (2.0 M in IPA), -20 °C; (ii) TFAA, pyridine, THF, 0 °C; (iii) BBr_3, DCM, -78 °C→rt.

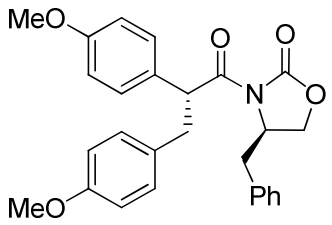
(*R*)-4-Benzyl-3-(2-(4-methoxyphenyl)acetyl)oxazolidin-2-one (11).



To a mixture of 4-methoxyphenylacetic acid (**4**, 3.77 g, 22.7 mmol) and (*R*)-(+)-4-benzyl-2-oxazolidinone (**10**, 2.01 g, 11.3 mmol) in $PhCH_3$ (36 mL) at room temperature was added triethylamine (6.32 mL, 45.4 mmol). The clear solution was heated to 80 °C for 10 min and then a solution of pivaloyl chloride (2.80 mL, 22.8 mmol) in $PhCH_3$ (4.7 mL) was added dropwise. After full addition, the reaction mixture was refluxed for 14 h before being cooled to room temperature and quenched with 1 M HCl (20 mL), extracted with EtOAc (2 X 50 mL), and the combined organic extracts were washed with 5% $NaHCO_3$ solution (15 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography (Hex:EtOAc, 2:1, to Hex:EtOAc:MeOH, 1:1:0.1) and recrystallization ($PhCH_3$:Hex, 1:1) afforded **11** (2.45 g, 66.8%) as a light yellow solid; mp 82-84 °C. R_f = 0.37 (Hex:EtOAc, 2:1). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.31-7.22 (m, 5H), 7.13 (d, *J* = 6.6 Hz, 2H), 6.88 (d, *J* = 8.8 Hz, 2H), 4.69-4.61 (m, 1H), 4.30-4.13 (m, 4H), 3.79 (s, 3H), 3.25 (dd, *J* = 13.3, 3.2 Hz, 1H), 2.74 (dd, *J* = 13.4, 9.5 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.6, 158.8, 153.4,

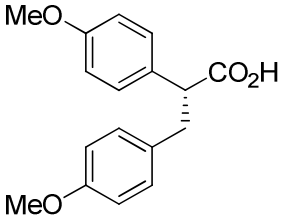
130.8, 129.4, 128.9, 127.3, 125.4, 122.5, 114.0, 66.1, 55.3, 55.2, 40.7, 37.1. HRMS (ESI) calc'd for $C_{19}H_{20}NO_4$ [M + 1] 326.1392; found 326.1393.

(*R*)-4-Benzyl-3-((*R*)-2,3-bis(4-methoxyphenyl)propanoyl)oxazolidin-2-one (12).



To a solution of **11** (2.51 g, 7.71 mmol) in THF (50 mL) at -78 °C was added NaHMDS (1.0 M in THF, 8.48 mL, 8.48 mmol) dropwise and left to stir at this temperature for 1 h. 4-Methoxybenzyl bromide (**7**, 2.24 mL, 15.4 mmol) was then added at -78 °C dropwise and left to stir to room temperature over 5 h before being quenched with H_2O (100 mL). The crude reaction was extracted with EtOAc (2 X 100 mL), and the combined organic extracts were dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography (Hex:EtOAc, 3:1) and recrystallization (Hex:EtOAc, 1:1) afforded **12** (2.58 g, 75.1%, dr >99:1) as a white solid; mp 169-170 °C. R_f = 0.49 (Hex:EtOAc, 2:1). $[\alpha]_D^{23}$ -144.1 (*c* 1.1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36 (d, *J* = 8.8 Hz, 2H), 7.28-7.21 (m, 5H), 7.17 (d, *J* = 8.6 Hz, 2H), 6.85 (d, *J* = 8.8 Hz, 2H), 6.80 (d, *J* = 8.6 Hz, 2H), 5.38 (dd, *J* = 9.8, 5.9 Hz, 1H), 4.61-4.52 (m, 1H), 4.04-3.99 (m, 2H), 3.79 (s, 3H), 3.75 (s, 3H), 3.44 (dd, *J* = 13.5, 9.6 Hz, 1H), 3.04-2.93 (m, 2H), 2.59 (dd, *J* = 13.5, 8.8 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.7, 158.9, 158.1, 152.8, 135.0, 131.1, 130.3, 130.3, 129.7, 129.4, 128.8, 127.2, 114.0, 113.7, 65.5, 55.3, 55.2, 55.2, 49.6, 39.7, 37.5. HRMS (ESI) calc'd for $C_{27}H_{28}NO_5$ [M + 1] 446.1967; found 446.1967.

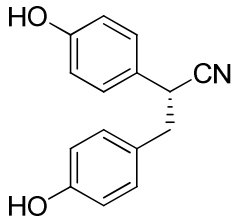
(*R*)-2,3-bis(4-methoxyphenyl)propanoic acid (13).



To a solution of **12** (1.01 g, 2.27 mmol) in THF:H_2O (120 mL, 5:1) at 0 °C was added H_2O_2 (30% wt in H_2O, 14.3 mL) and LiOH (54.3 mg, 2.27 mmol). The resulting white suspension was stirred at 0 °C for 3 h before being quenched with cold 0.1 M HCl (20 mL). The residue was extracted with EtOAc (2 X 100 mL), and the combined organic extracts were dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography (EtOAc:Hex, 2:1) afforded **13** (0.64 g, 98.4%) as an off-white solid; mp 118-120 °C. R_f =

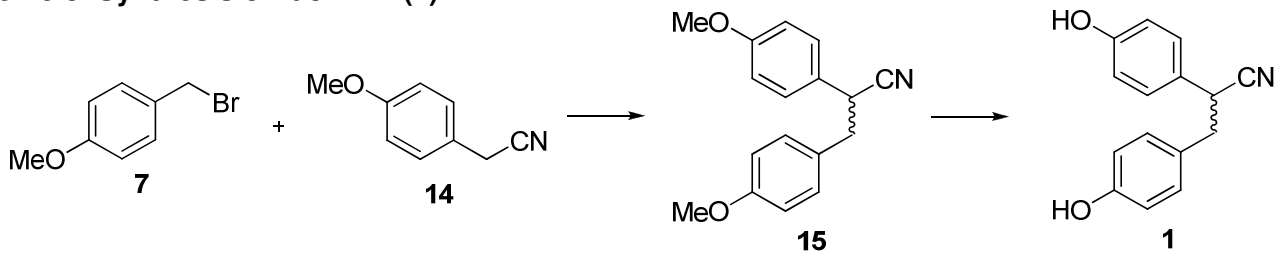
0.53 (Hex:EtOAc, 1:1). $[\alpha]_D^{23}$ -137.6 (*c* 1.0, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.22 (d, *J* = 8.8 Hz, 2H), 7.02 (d, *J* = 8.3 Hz, 2H), 6.85 (d, *J* = 8.3 Hz, 2H), 6.77 (d, *J* = 8.3 Hz, 2H), 3.83-3.70 (m, 7H), 3.32 (dd, *J* = 13.9, 8.3 Hz, 1H), 2.96 (dd, *J* = 14.0, 7.2 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 179.1, 158.9, 158.1, 130.8, 130.0, 129.9, 129.1, 114.0, 113.7, 55.2, 55.2, 52.7, 38.4. HRMS (ESI) calc'd for $C_{17}H_{18}NO_4Na$ [M + 1] 309.1103; found 309.1108.

(*R*)-2,3-Bis(4-hydroxyphenyl)propanenitrile (3).

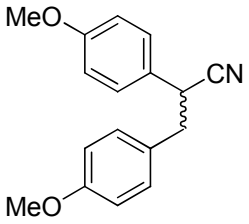


To a solution of **13** (50.1 mg, 0.18 mmol) in triethylamine (36.6 µL, 0.26 mmol) and THF (4 mL) at -20 °C was added isobutyl chloroformate (45.5 µL, 0.35 mmol). The resulting solution was stirred for 20 min at -20 °C, followed by the addition of ammonia (2.0 M in IPA, 0.88 mL, 1.75 mmol) and left to stir for an additional 20 min at -20 °C before being quenched by passing through a Celite plug. The crude solution was concentrated *in vacuo*, redissolved in THF (1.5 mL) and cooled to 0 °C, followed by addition of pyridine (60.9 µL, 0.75 mmol) and trifluoroacetic anhydride (51.1 µL, 0.37 mmol). The mixture was left to stir at 0 °C for 5 min before being quenched passing through a Celite plug and evaporation of solvent. The crude was redissolved in DCM (1.5 mL), cooled to -78 °C and BBr_3 (1.0 M in DCM, 1.5 mL, 1.50 mmol) was added dropwise over 5 min. The resulting mixture was left to warm to room temperature over 3 h before being quenched upon slow addition of MeOH at 0 °C. The crude solution was passed through a Celite plug, concentrated *in vacuo* and recrystallized (Hex:EtOAc, 1:1) to afford **3** (24.3 mg, 58.1% over 3 steps) as an off-white solid; mp 190-192 °C. R_f = 0.45 (Hex:EtOAc, 1:1). $[\alpha]_D^{23}$ -1.355 (*c* 1.1, MeOH). ^{1}H NMR (500 MHz, CD_3CN) δ 7.15 (d, *J* = 8.5 Hz, 2H), 7.03 (d, *J* = 8.5 Hz, 2H), 6.80 (d, *J* = 8.5 Hz, 2H), 6.73 (d, *J* = 8.5 Hz, 2H), 4.07 (t, *J* = 7.7 Hz, 1H), 3.09-2.97 (m, 2H). ^{13}C NMR (126 MHz, CD_3CN) δ 157.7, 157.0, 131.5, 129.9, 129.5, 128.2, 122.2, 116.6, 116.1, 41.4, 39.4. HRMS (ESI) calc'd for $C_{15}H_{13}NO_2Na$ 262.0844; found 262.0846.

Scheme 3. Synthesis of *rac*-DPN (1)

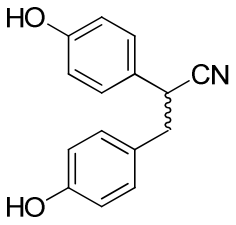


2,3-Bis(4-methoxyphenyl)propanenitrile (15).



To a solution of 4-methoxyphenylacetonitrile (**14**, 1.01 g, 6.86 mmol) in THF (10 mL) at 0 °C was added NaH (60% dispersion in oil, 0.30 g, 7.55 mmol) and left to stir at this temperature for 20 min, followed by the addition of 4-methoxybenzyl bromide (2.00 mL, 13.7 mmol) dropwise. The resulting mixture was left to warm to room temperature overnight before being quenched with H_2O (30 mL). The residue was extracted with EtOAc (2 X 50 mL), and the combined organic extracts were dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography (Hex:EtOAc, 4:1) afforded **15** (1.45 g, 79.3%) as a white solid; mp 113-115 °C. R_f = 0.75 (Hex:EtOAc, 2:1). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.15 (d, *J* = 8.8 Hz, 2H), 7.03 (d, *J* = 8.6 Hz, 2H), 6.87 (d, *J* = 8.8 Hz, 2H), 6.82 (d, *J* = 8.8 Hz, 2H), 3.91 (dd, *J* = 8.0, 6.5 Hz, 1H), 3.81 (s, 3H), 3.79 (s, 3H), 3.15-3.00 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 159.3, 158.8, 130.3, 128.6, 128.4, 127.2, 120.7, 114.3, 113.9, 55.3, 55.2, 41.5, 39.2. HRMS (ESI) calc'd for $C_{17}H_{17}NO_2Na$ 290.1157; found 290.1158.

2,3-Bis(4-hydroxyphenyl)propanenitrile (1).



To a solution of **15** (0.49 g, 1.81 mmol) in DCM (20 mL) at -78 °C was added BBr_3 (1.0 M in DCM, 14.5 mL, 14.5 mmol) dropwise. The resulting mixture was allowed to warm to room temperature over 3 h before

being quenched at 0 °C with MeOH, followed by the addition of H_2O (20 mL). After the effervescence ceased, the crude solution was extracted with EtOAc (2 X 30 mL), and the combined organic extracts were dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography (Hex:EtOAc, 1:1) afforded **1** (0.36, 82.1%) as a white solid; mp 197-199 °C. R_f = 0.15 (Hex:EtOAc, 2:1). ^{1}H NMR (500 MHz, CD_3CN) δ 7.14 (d, *J* = 8.3 Hz, 2H), 7.02 (d, *J* = 8.5 Hz, 2H), 6.79 (d, *J* = 8.5 Hz, 2H), 6.72 (d, *J* = 8.5 Hz, 2H), 4.09-4.02 (m, 1H), 3.07-2.97 (m, 2H). ^{13}C NMR (126 MHz, CD_3CN) δ 157.7, 157.0, 131.5, 129.9, 129.5, 128.2, 122.2, 116.6, 116.1, 41.4, 39.4. HRMS (ESI) calc'd for $C_{15}H_{13}NO_2Na$ 262.0844; found 262.0842.

Chiral HPLC Conditions:

Column: Regis Technologies (R,R)-Whelk-O® 2 column (Particle Size: 10 µm, 100 Å, Column Dimensions: 25 cm X 4.6 mm, Cat. #: 786315).

Conditions:

Solvent: 80% Hexanes 20% Isopropyl Alcohol

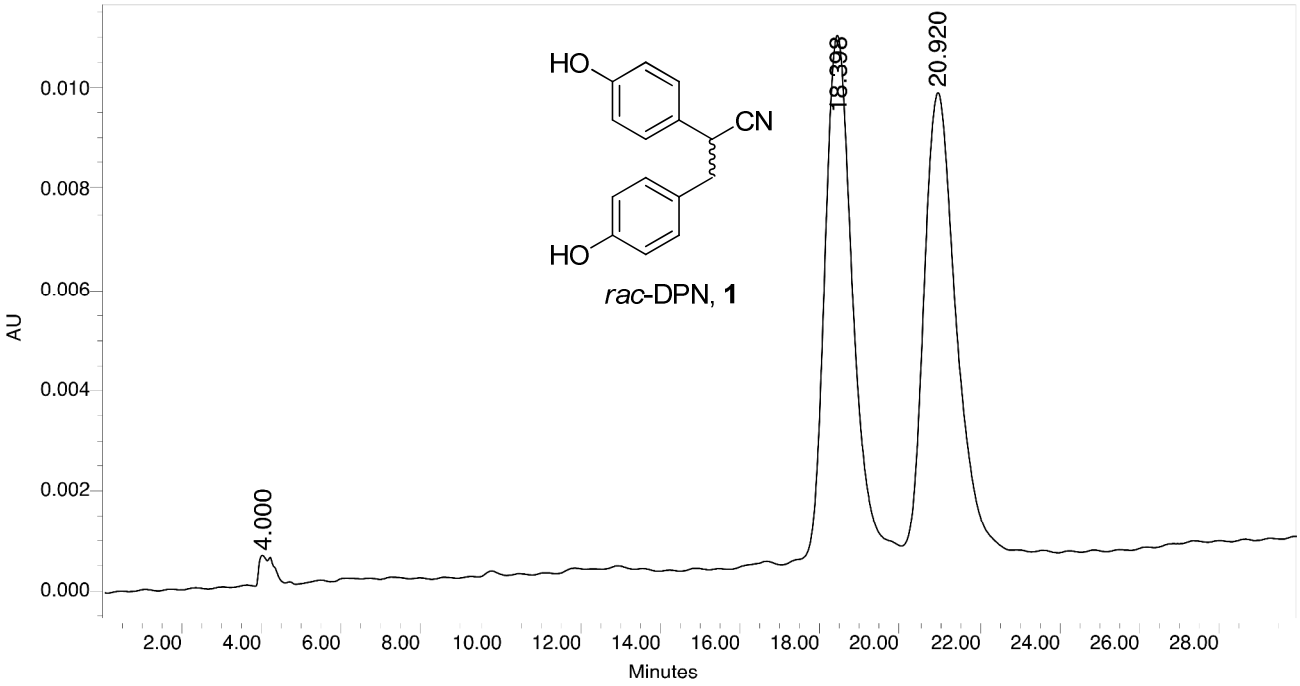
Flow Rate: 0.8 mL/min (isocratic)

λ: 254 nm

U of IL
Project Name Defaults
Reported by User System

SAMPLE INFORMATION

Sample Name	VMC-03-DPN-OH-rac	Acquired By:	System
Sample Type	Unknown	Date Acquired	6/4/2010 10:16:34 AM
Vial:	3	Acq. Method	VMC_80_20_H_IPA
Injection#:	1	Date Processed	6/28/2010 12:54:01 PM
Injection Volume	10.00 ul	Channel Name	2487Channel1
Run Time	30.00 Minutes	Sample Set Name	



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1	4.000	14344	1.43	589	2.94
2	18.398	495081	49.44	10377	51.81
3	20.920	492016	49.13	9064	45.25

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Project Name Defaults
Reported by User System

SAMPLE INFORMATION

Sample Name	VMC03-S-DPNOH	Acquired By:	System
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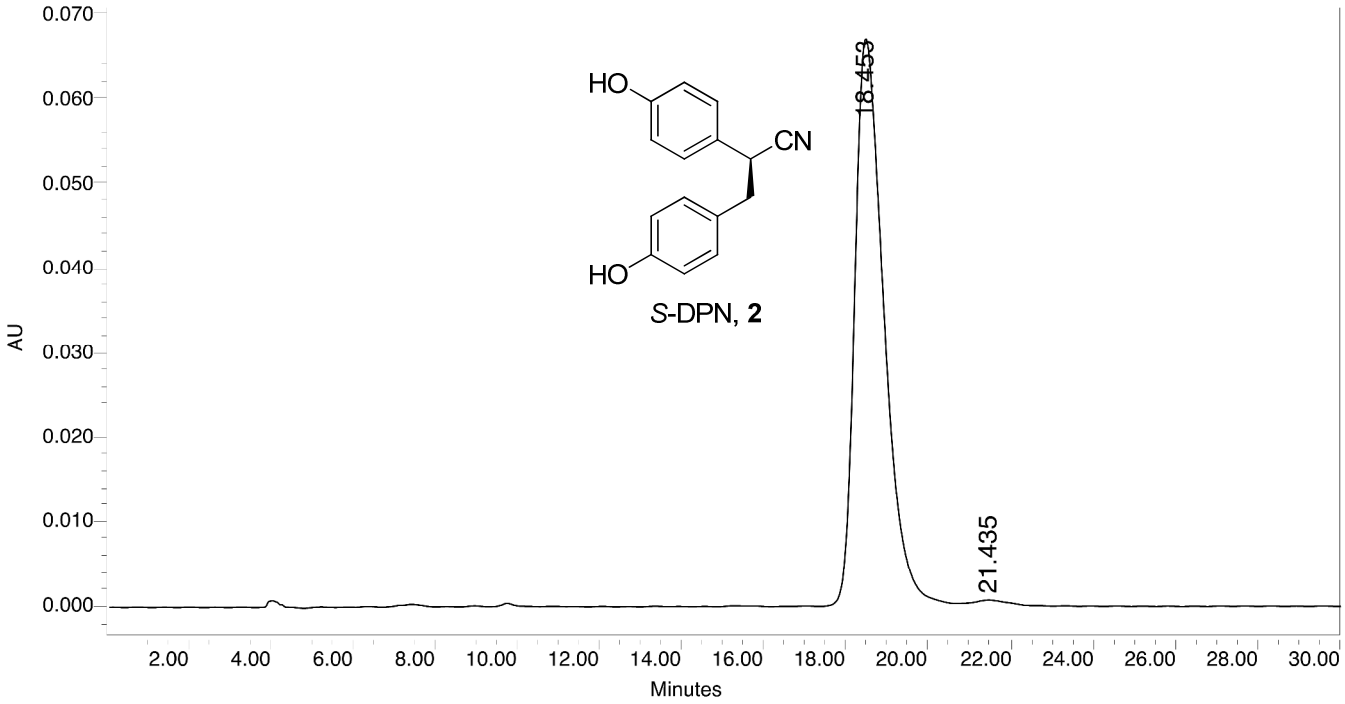
Vial:	9	Acq. Method	VMC_80_20_H_IPA
Injection#:	1	Date Processed	6/28/2010 12:59:37 PM
Injection Volume	10.00 ul	Channel Name	2487Channel1
Run Time	30.00 Minutes	Sample Set Name	



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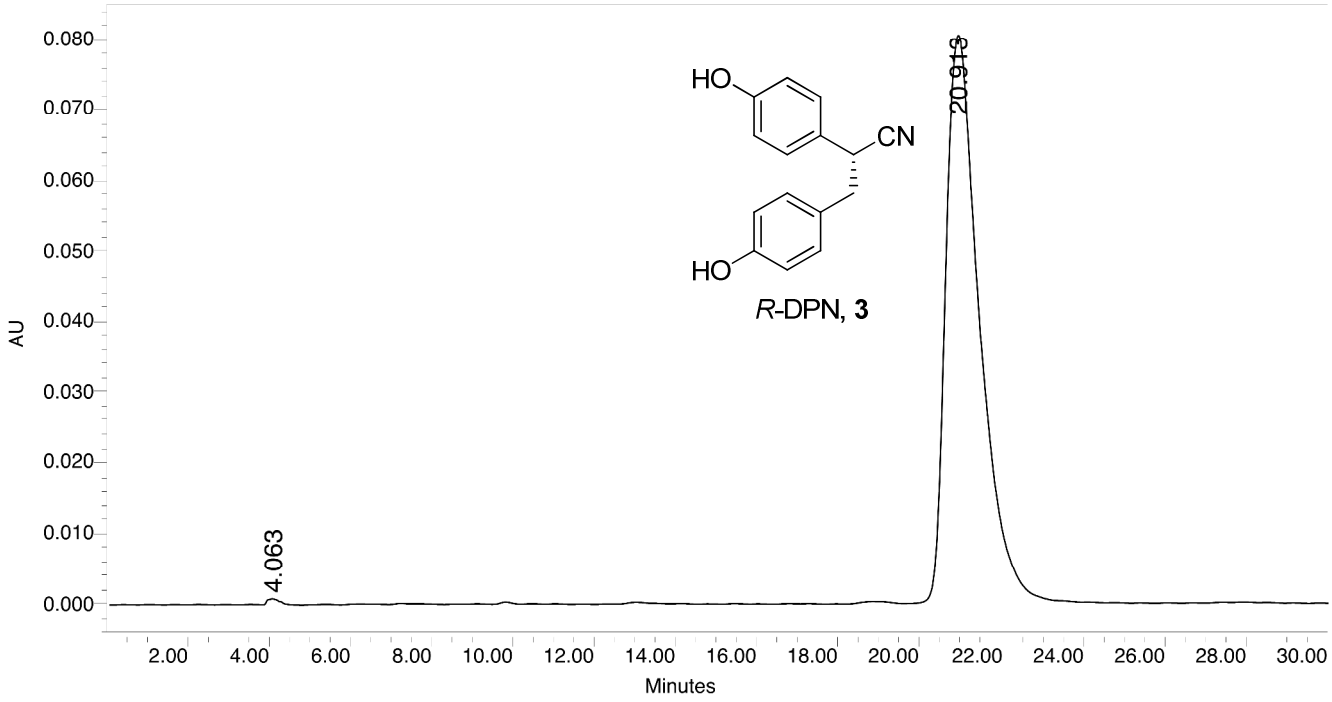
Project Name Defaults
Reported by User System

SAMPLE INFORMATION

Sample Name	VMC03-R-DPNOH	Acquired By:	System
Sample Type	Unknown	Date Acquired	6/5/2010 12:20:35 PM
Vial:	10	Acq. Method	VMC_80_20_H_IPA
Injection#:	1	Date Processed	6/28/2010 12:53:19 PM
Injection Volume	10.00 ul	Channel Name	2487Channel1
Run Time	30.00 Minutes	Sample Set Name	



	RT (min)	Area (V *sec)	% Area	Height (V)	% Height
1	4.063	17396	0.38	838	1.03
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